

16.

Correlations Between Structural Eye Defects and Behavior in the Mexican Blind Characin.

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(Plates I-IV; Text-figures 1 & 2).

INTRODUCTION.

Hubbs & Innes (1936) reported the discovery of a new species of blind cave fish inhabiting the warm water caves in the vicinity of Pujal, San Luis Potosi, Mexico, and named it *Anoptichthys jordani*. This form is genetically continuous with *Astyanax mexicanus* (Fillipi) from which it is distinguishable only in the undeveloped eye and lack of pigmentation. The eye structure of these fish was found to be imperfectly developed and morphologically arrested at a low level of embryological life without powers of visual function, Gresser & Breder (1940).

Especial interest in this species of fish lies in the fact that it is the only known cave species with clear genetic continuity to an outside visual type. Inhabiting warm water and being suitable for laboratory culture, it predicates an experimental approach to problems of blind cave fauna that have vexed biologists. The Aquarium Cave Expedition, Bridges (1940), established this genetic continuity and prepared the bases for further study. It was at first considered that the native habitat could best be utilized for the purpose with the establishment of controlled breeding tanks within the cave water system, but practical considerations caused an abandonment of such an approach.

La Cueva Chica is roughly eighty miles due west of Tampico and some 350 miles northeast of Mexico City, close to the Pan-American Highway at the village of Pujal. Running nearly due south about 1,000 feet from its entrance, the cave ends in a large cul-de-sac five-eighths of a mile from the Rio Tapaon. The character of the cave water system, its flow and the presence of sink-holes in the probable line of direction between cave and river bespoke a close connection, if not a direct one, between these two

systems. Within the cave there were four major pools of water, the innermost of which at the time of our visit was isolated as far as could be determined. The remaining three pools were clearly connected by a small stream. See Bridges (1940) for a map of the cave.

Our thanks are due Mr. James W. Atz for his patient assistance in making a considerable portion of the observations listed in Table III.

DESCRIPTIVE ANATOMY.

All the pools were seined to determine the character of the inhabitants. Grossly there were found three fairly distinct groups, one of which was divisible into two subdivisions. The somewhat arbitrary groups follow: (1) obviously blind with deformed, undeveloped eyes; (2) fish with sunken eyes, (a) covered by skin epithelium, (b) not covered by skin; (3) normal eyes. The sampling is given in Table I. Histological studies were made by serial sections covering a considerable amount of the catch. Variations within the groups as to extent of development both grossly, histologically and to absolute size were numerous.

Group 1 has been previously described in detail, Gresser & Breder (1940), and consists generally of an ocular sac connected to the skin surface by a connective tissue strand either solid or cystic and lined with epithelium. The ocular structures present are defective as to elements and form, the retinal vesicle usually being a lobulated sac without differentiation into iris, pupillary space or developing sensory elements. A crystalline lens is absent and the anterior chamber is absent or represented by an unlined space in a connective tissue stroma. The optic nerve is represented chiefly by a connective tissue cord. See Plate I.

In group 2a the covered, sunken eye presents a better developed globe although malformed as to size and shape. The choroidal gland is usually well developed and the retina is either completely deficient in sensory elements and ganglion cells or large areas of retina are so deficient. A crystalline lens when present is smaller than normal and is accompanied by an irregularly oval-shaped pupillary space; where absent the iris is a weak membrane forming a complete anterior wall to the retinal vesicle. In such conditions an extremely shallow anterior chamber can be found. See Plate II.

The uncovered, sunken eye, group 2b, varies greatly in detail but generally is of small size, deformed but with an anterior chamber, an iris quite well developed with a pupillary space. A lens is present but smaller in diameter in relation to globe diameter than normal. The retina is better stratified than in previous types but deficient irregularly in the rod-cone layer and the corresponding ganglion cell layer. The optic nerve reflects this deficiency in size and relative absence of nerve fibers. See Plate III.

The orbital cavity in group 2 is well developed and where the globe is small or deformed there is a corresponding increase in orbital fat. The extraocular muscles are intact in all groups but apparently their functional use is limited in accordance with the functional capacity of the eye.

In the specimens examined, no symmetry of binocular development could be determined in individuals of groups 1 and 2; especially in group 2 asymmetry was marked as to morphological and functional development. Group 3 was indistinguishable from the normal river fish but microphthalmia was frequent. The normal typically piscine eye structure is shown in Plate IV, based on a specimen from the Rio Tampaon.

Obviously the pool formations present formed ideal and natural breeding tanks and presented *in loco* a highly suggestive explanation for the eye defects and probably as superior and factual experimental methods as human endeavor could devise and control.

As was noted above, population studies in the most isolated pool No. 1 showed a nearly pure strain with completely ineffectual eye structures with an increase in percentage of better developed ocular organs as the probable connection with the outside stream was approached, so that in pool No. 4, closest to the Rio Tampaon, were contained a large percentage of structurally normal eyes.

Most of the inhabitants of pool No. 1 conformed to the ocular pattern first described and which originally were obtained from this pool. Inhabitants of pool No. 2 showed a small percentage of intermediate eyes in which variations in size of the globe, pupillary openings and tissue structures were found but none of which presented morphologically an image-forming organ. In pool No. 3 intermediate eyes were found in greater proportion. In this pool occasional inhabitants showed normally sized eyes, struc-

turally well developed and which clearly permitted definite light behavior patterns. These fish also showed a greater amount of skin pigmentation. This data is detailed in Tables I and II.

Of the inhabitants seined from pool No. 4, only occasional specimens were of least development with a preponderance of better-developed eyes and an increase in the proportion of normals both as to eye structure and pigmentation.

TABLE I.

Eye Condition and Pigmentation of Cave Characins. Expressed in % of catch. Based on 119 specimens.

Eye Condition					
Location	Blind (1)	Sunken Eye (2)		"Normal Eye" (3)	
		Covered (a)	Uncovered (b)		
Pool 2	85	6	—	9	
Pool 3	16*	8	45	31	
Pool 4	—	9	9	82	

Pigmentation					
Location	None	Little	Moderate	Considerable	Full
Pool 2	90	2	6	2	—
Pool 3	34*	34	5	8	19
Pool 4	3	29	32	24	12

* Two specimens in this group blind on one side only.

TABLE II.

Association of Eye Condition and Extent of Pigmentation.

Expressed in % of extent of pigmentation. Based on 119 specimens.

Eye Condition	Extent of Pigmentation in Per Cent.				
	None	Little	Moderate	Considerable	Full
Blind (1)	98*	—	2	—	—
Sunken Eye (covered) (2a)	56	33	11	—	—
Sunken Eye (uncovered) (2b)	—	62	15	15	8
"Normal" (3)	2	23	28	23	24

* Two specimens in this group blind on one side only.

BEHAVIOR EXPERIMENTS.

Fishes representing the various types described in the preceding section were shipped alive to the New York Aquarium and form the bases on which the following experiments were made. In addition to these, specimens of the fully blind type which had been bred to the fifth generation by Mr. Albert Greenberg of Tampa, Florida, were used for comparative purposes. These had all been reared in brilliant light. Since Mr. Greenberg never obtained any but fully blind fish it is evident that this form breeds true to type.

Even casual observation shows that these optically various fishes present different types of locomotor behavior. The fully blind individuals, both direct from the cave and Greenberg's fifth generation fish, all keep up a continual swimming activity, constantly wandering in a seemingly

aimless manner. Even at night, if a light were suddenly struck, they would be found to be moving about, giving the impression that they managed to keep on the move at all times. The normal river fish, on the other hand, unless feeding or engaged in some other activity, would remain quiescent in a school, only moving under some evident external stimulus. Those with intermediate eyes unable to form a retinal image but clearly able to receive an optical stimulus from a light source, behaved like the fully blind while those forming a defective image behaved like the normally eyed fish.

Normally eyed fish brought from the cave at first acted nearly like the blind groups but later took on the habits of the typical river fish. It would thus appear that these fishes, unaccustomed to retinal images, took an appreciable time to be able to react to them in an appropriate fashion.

In order better to understand the relation of eye defect to behavior as measured by their locomotor habits, troughs were established

having a lighted and a dark end. These were so arranged that a given area was illuminated with a 60-watt bulb at a distance of three feet while the remainder was shadowed by a cover close to the surface of the water. Thus it was anticipated that by noting the positions of the fish in unit intervals their preferences could be determined in a quantitative manner. In the case of a randomly wandering fish the number of observations obtained over a given area of bottom should then be directly proportional to the whole area if there were no preference being expressed. Significant deviations from such a figure would then be a measure of preference.

As a preliminary experiment a trough was set up 1' by 4' with water 6" deep. Three feet of its length was covered; the remaining area was exposed to light. In all cases the entire tanks were painted black in order to minimize reflections. Into this were placed four fully blind fish of the fifth generation. If these fish were moving strictly at random it then follows that the average of the unit observations which were made

TABLE III.
Results of Behavior Experiments.

% of						% of						% of		
Exp. No.	Area	No. of Obs.	Fish No.	Type	Obs. in Light	Random Expectation	Exp. No.	Area	No. of Obs.	Fish No.	Type	Obs. in Light	Random Expectation	
1	.25	100	4	5th Generation	73	73	42	.50	100	1	" "	37	74	
2	.25	100	4	" "	111	111	43	.50	100	1	" "	36	72	
3	.25	100	4	" "	88	88	44	.50	100	1	" "	29	58	
4	.25	100	4	" "	54	54	45	.50	100	1	" "	15	30	
5	.25	100	4	" "	96	96	46	.50	100	1	" "	55	110	
6	.25	100	4	" "	81	81	47	.50	100	1	" "	46	92	
7	.25	100	4	" "	45	45	48	.50	100	3	" "	133	88.6+	
8	.25	100	4	" "	55	55	49	.50	100	3	" "	114	76	
9	.25	100	4	" "	85	85	50	.50	100	1	No Image Cave	43	86	
10	.25	100	4	" "	67	67	51	.50	100	1	" " "	42	84	
11	.50	100	4	" "	131	65.5	52	.50	100	1	" " "	11	22	
12	.50	100	4	" "	133	66.5	53	.50	100	1	" " "	26	52	
13	.50	100	2	" "	29	29	54	.50	100	1	" " "	21	42	
14	.50	100	2	" "	35	35	55	.50	100	1	" " "	11	22	
15	.50	100	2	" "	24	24	56	.50	100	1	" " "	73	146	
16	.50	100	1	" "	42	84	57	.50	100	1	" " "	87	174	
17	.50	100	1	" "	23	46	58	.50	100	1	" " "	7	14	
18	.50	100	1	" "	24	48	59	.50	100	1	" " "	12	24	
19	.50	100	1	" "	12	24	60	.50	100	1	" " "	21	42	
20	.50	100	1	" "	28	56	61	.50	100	1	" " "	21	42	
21	.50	100	1	" "	37	74	62	.50	100	1	" " "	31	62	
22	.25	100	2	Blind Cave	42	84	63	.50	100	1	" " "	25	50	
23	.25	100	2	" "	61	122	64	.50	100	1	" " "	23	46	
24	.50	75	1	" "	30	80	65	.50	100	1	" " "	26	52	
25	.50	100	1	Blind Cave	34	68	66	.50	100	1	Image Cave	48	96	
26	.50	100	1	" "	50	100	67	.50	100	1	" " "	3	6	
27	.50	100	1	" "	51	102	68	.50	100	1	" " "	3	6	
28	.50	100	1	" "	48	96	69	.50	100	1	" " "	0	0	
29	.50	100	1	" "	40	80	70	.50	100	1	" " "	0	0	
30	.50	125	1	" "	17	27.2	71	.50	100	1	" " "	0	0	
31	.50	100	1	" "	37	74	72	.50	100	1	" " "	0	0	
32	.50	100	1	" "	38	76	73	.50	100	1	" " "	0	0	
33	.50	100	1	" "	27	54	74	.50	100	1	" " "	0	0	
34	.50	100	1	" "	31	62	75	.50	100	1	" " *	9	18	
35	.50	100	1	" "	53	106								
36	.50	100	1	" "	21	42					Maximum	174		
37	.50	100	1	" "	44	88					Average	61.4	—	
38	.50	100	1	" "	43	86					Minimum	0		
39	.50	100	1	" "	45	90								
40	.50	100	1	" "	51	102								
41	.50	100	1	" "	46	92								

* Not the same specimen used in Experiments 66 to 74.

at five second intervals should come to the figure "1." That is, on strictly random distribution there should be three fish in the dark area and one in the light, or if the fish were schooling there should be four fish visible on the average every fourth observation. In a series of such tests, in which there were 2,100 such observations, the percentage of expectation was 62.3-% in light. This data is given in detail in Table III. Since experiments consist of 100 observations, the expectation from random movement should give 100%. Only one observation reached that figure. As the illuminated area of the trough was changed from time to time in order to avoid the inclusion of some other possible but unknown factor, it can only be concluded that by some means these fish were light sensitive to a slight degree and reacted negatively to such radiations or some secondary associated effect.

Subsequent observations were made in smaller troughs 1' x 2' x 6" deep in which one-half was illuminated and one-half in darkness. Here fish in various smaller numbers were similarly checked and found to show like reactions, as is also set forth in Table III.

Other fishes were then checked in a similar manner and these data are also given in Table III. They are summarized and presented in a condensed form in Table IV. From these tables it is evident that the fully blind fish brought from La Cueva Chica reacted in a manner similar to those of the fifth generation reared in light: 80% avoidance as against 62%. This difference of 17% may actually be significant. The cave fish were larger than the others and possibly overlying tissues of greater consequent thickness may account for the difference, or it may be that there is an increased avoidance to light in subsequent generations.

TABLE IV.

Averages and Ranges of Experiments.

Exp. No.	Type of Fish	No. of Obs.	% of Random Expectation		
			Max.	Mean	Min.
1 to 21	5th Generation	2,100	111	62.3	— 24
22 to 49	La Cueva Chica (Blind)	2,800	122	79.6	+ 27
50 to 65	La Cueva Chica (No image)	1,600	154	58.7	+ 14
66 to 74	La Cueva Chica (Image)	900	96	12.0	0
75	La Cueva Chica (Image)	100	—	18.0	—
—	River Fish (Normal)	—	Fully unreadable		

Experiments 50 to 65 were performed on a fish that could distinguish light but not form an image. Fish of this group, too, avoided light, 59%, even more vigorously, but here was clear optical detection. Whether or not these fish form a useful retinal image is easy to detect. Fully blind ones, although feebly light-sensitive in the above sense, will not respond to a shadow intercepting the light falling on a tank. Those with full or partial vision react violently by dashing about when a shadow passes across them.

The feeding reactions separate the latter two types. Those with image formation will come to the surface or near it, roll their eyes and make energetic strikes at the food particles as they sink through the water. The blind or merely light-detecting individuals react in no such fashion, merely cruising about mostly on the bottom and apparently finding their food by energetic random movement. One individual seemed to be just at the border line of image formation, normally cruising about like the blind but occasionally finding a sinking particle if it fell between the fish and the light source.

Experiments 66 to 74 were performed on a fish with defective eyes but which could evidently form some sort of image. Experiment 75 was performed on another specimen of similar condition. Here the percentage of expectation of the fish in the light was clearly much lower than in any of the others. Actually these fish spent most of their time just under the shelter of the shadow, obviously looking out into the lighted area, not wandering about but resting quietly.

TABLE V.

Aggregation Data on Blind Specimens.

Exp. No.	Nos. of observations of fish by groups in lighted area				
	0	1	2	3	4
1	48	35	14	2	1
2	34	31	26	8	1
3	38	37	24	1	0
4	52	42	6	0	0
5	32	46	17	4	1
6	43	37	16	4	0
7	62	31	7	0	0
8	59	28	12	1	0
9	35	48	14	3	0
10	44	44	11	1	0
Average	44.7	37.9	14.7	2.4	0.3
11	18	46	24	11	1
12	17	41	34	8	0
Average	17.5	43.5	29.0	9.5	0.5
13	73	24	3		
14	71	23	6		
15	79	18	3		
Average	74.0	27.0	4.0		
22	65	28	7		
23	48	43	9		
Average	56.5	36.5	8.0		
48	16	42	35	7	
49	19	48	32	1	
Average	17.5	45.0	33.5	4.0	

Comparison with Theoretical Probability.

4 fish ¼ area	31.6	42.2	21.1	4.7	0.4
Exp. 1-10	44.7	37.9	14.7	2.4	0.3
4 fish ½ area	6.25	25.0	37.5	25.0	6.25
Exp. 11-12	17.5	43.5	29.0	9.0	0.5
3 fish ½ area	12.5	37.5	37.5	12.5	
Exp. 48-49	17.5	45.0	33.5	4.0	
2 fish ½ area	25.0	50.0	25.0		
Exp. 13-15	74.0	27.0	4.0		
Exp. 22-23	56.5	36.5	8.0		

Normal river fish acted much as the above, but stayed in the light continually unless disturbed by a slight noise or jar to the building. Consequently they were unreadable by this technique since their movements were controlled by external stimuli which were, so far as possible, prevented from reaching them during the course of the experiments.

Since the normal river fish are a closely aggregating and to a considerable extent a schooling type, the annotations previously discussed were examined in reference to the appearance of groups of blind individuals where more than one fish was used in an experiment. These groups were used primarily to discover if any social influences were at work in regard to the light-avoiding reaction. Nothing of this sort appeared.

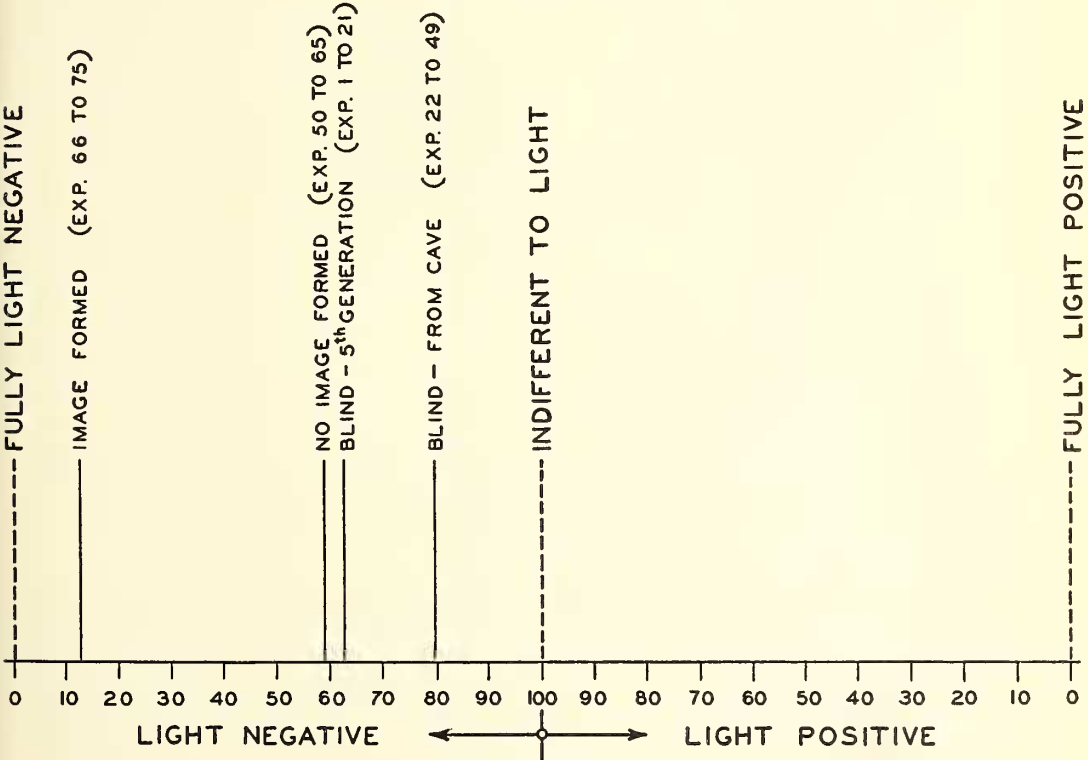
Since it is evident that the seeing forms show a strong aggregating tendency, the data accumulated on the blind specimens were analyzed so as to show evidences of similar behavior in these fishes if such were present. The data are given in Table V and shown graphically in Text-figure 1 which indicates the results of these tests in reference to random expectation.

As a definite number of fishes was used in each experiment and the lighted bottom area bore a definite relationship to the unlighted, $\frac{1}{4}$ and $\frac{1}{2}$,

in the two types of tanks used, it follows that if the fish were moving at random without reference to light or each other their occurrence in the lighted area in ones, twos, threes, etc., should be predictable according to the binomial formula $(p + q)^n$ expanded for each term, in which

- n = number of fishes
 p = lighted area
 q = dark area
- (1) p^n
- (2) $np^{n-1}q$
- (3) $\frac{n(n-1)}{1 \cdot 2} p^{n-2}q^2$
- (4) $\frac{n(n-1)(n-2)}{1 \cdot 2 \cdot 3} p^{n-3}q^3$
- (5) $\frac{n(n-1)(n-2)(n-3)}{1 \cdot 2 \cdot 3 \cdot 4} p^{n-4}q^4$

These calculations are given in Table V for comparison with the observed figures for the several types of experiment. Some rather interesting features appear which are more clearly shown in Text-figure 2 which presents the comparative data graphically. It may be seen in the



Text-figure 1.

Comparison of behavior in various experiments on light sensitivity in terms of expectancy of random movement.

data of Table V and in Text-figure 2 that in each case there were more observations of the lighted area without fish than random expectation calls for, which is another way of measuring these fishes' light avoidance. In Text-figure 2, graph A, it is clear that the occurrence of the fish in the lighted area in groups follows closely that of the theoretical probability. All appear as less than the expected random value, which again is a measure of their individual light avoidance; the slopes of the connecting lines are in close agreement with random expectation. This is the measure of interlocked (aggregating) behavior and is clearly shown to be a good experimental zero. This graph is based on the largest number of experiments and shows closer agreement with the theoretical than the others based on smaller numbers.

In graph B of Text-figure 2 both the observations noting "no fish" and "one fish" are greater than random expectation while the rest are below it. This is also true for graph C. Except for this feature they are in agreement with graph A although based on much less data. Graph B represents the same fish (fifth generation) as in A while C represents fish brought from the cave. Whatever the interpretation placed on this, these two groups were showing practically identical behavior. It is suspected that the small number of experiments, 3, give as good approximation as could be expected. This becomes more apparent when graph D is considered. Here two sets of different fish show close agreement with A, the "O" observations being above random expectation and the rest below with the trend of these lines being very nearly parallel. It can only be concluded that these fish were moving strictly at random in reference to each other but with a marked avoidance of the lighted area.

DISCUSSION.

From the preceding descriptive histological data, the reactions of the various types with more or less defective vision, coupled with the experimental data on the avoidance of light and the social attitude of the blind examples, certain features become apparent that should have great significance in the development of cave-dwelling blind fish.

If we operate from the assumption that blind and pigmentless animals are able to establish themselves in caves because therein both eyed and eyeless forms are on equal terms, we have two basic factors in effect and interacting; a genetic defect and an environmental peculiarity, the presence of the latter making a continuity and establishment of the former possible. In addition to these it is here experimentally shown that there is a third, a behavior factor, which operates in a positive way.

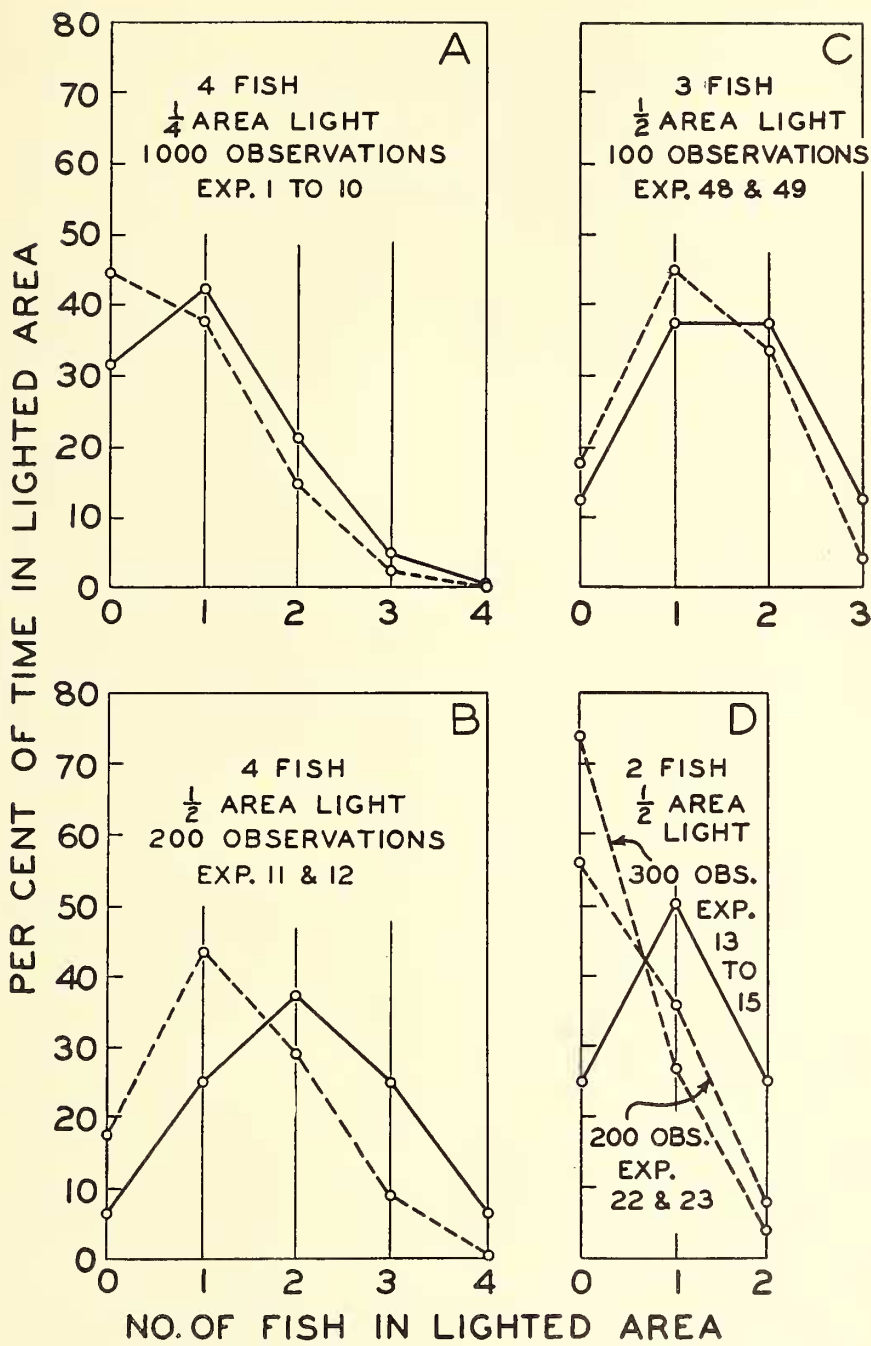
Since the eyed forms use dark retreats only under the stimulus of fright and the blind ones avoid light, by a mechanism as yet not understood, we have a marked schism in the behavior

of fishes otherwise identical and of genetic continuity. Consequently it would seem fair to make the following assumptions on the operation of these factors in a state of nature.

Given a population of river fish carrying one or more appropriately defective genes, individuals with slightly defective sight would seek the shelter of rocks and crevices more than their normally eyed fellows. A cave from which water leaves (as La Cueva Chica) would encourage entry, as these fish tend to work their way up small streams (contranatant) and hold their position in the upper reaches of the rivers by this means. Selection might enter at this point, *i. e.*, fish with defective eyes that might happen to prefer the light would not stay or enter the cave and would either be lost or mixed with the river population, depending on the extent of the handicap of the visual defect. Those that happened to prefer the dark would stay there and transmit their defect, increasing and intensifying it by consanguinity. Once unable to form an image they would not seek light, and if they emerged at night would retreat with sunrise or be lost, as witness what happens in a lighted aquarium between image-forming and non-image forming cave individuals. It appears that the seeing fish attempting to school with the sightless cannot keep up with their continual aimless wandering. This leads to a "resentment" that frequently results in the eyed form attacking and killing the blind. There is no evidence of a substitute sensory mechanism having been developed in the blind fish, for the changing of the position of a rock in an aquarium will result in their swimming into it for a time. Apparently they merely learn where obstacles are by a trial and error development of some sense of space and distances. One specimen with image formation and miniature eyes was successfully kept with two fully blind individuals but took on their habits after about a month and showed no evidence of attack but spent much time following the blind ones. It is probably more than accidental that this fish exhibited great nervousness, dashing about wildly on approach to the aquarium. Returning to the discussion of natural conditions, it would seem that those which found their way out of the cave, and with good eyes, might stay out; however, the current tends to keep them in, as these fish will not normally drop down stream and the period necessary for them to develop an understanding of a retinal image might well be their undoing.

Thus the river and cave populations would tend to stay fairly separate with perhaps the continual entry of defective-eyed fishes accounting for the gradient of eyelessness within the cave.

The question of whether the present population was once fully cut off geographically from the river fish is rather pointless in the light of the above. Certainly some such condition as mentioned above was present at the time of original entry. If some geologic cataclysm cut off these fish for a time, they rejoined sufficiently soon to make a reestablishment of the original condition



Text-figure 2.

Comparison of theoretical randomness with actual behavior showing lack of schooling behavior and negative reactions to light in fully blind fish. Solid line: mathematical probability. Dotted line: observed behavior.

possible, for otherwise the gradient would be quite inexplicable.

If it is thought that some reestablishment of Lamarkian ideas are indicated, it may be pointed out that if the blindness were indeed induced by such a mechanism, the present data do nothing to establish it. Fish that have been forced to live in light for five generations are apparently even more "anxious" to get back into the cave than the present dwellers therein. If this means an increasing sensitivity to light (reestablishment of an eyed condition), it would defeat progress in that direction, for those in the process of regaining vision are the ones most anxious to avoid the stimulus inducing it. Such a mechanism should make for a status quo. A study of possible histological changes in the successive generations of those reared in light for a greater number of generations could help in this connection.

Consequently we are forced to the conclusion that the origin of this blindness is rooted in some genetic defect that was able to find expression in a lightless environment and that the differential behavior of those individuals able to form an optical image as opposed to those merely light sensitive is the determining factor in keeping the population of the cave from a depleting voluntary exit and from too great an admixture of the occasionally entering outside river fish with good vision. The details of the genetic ratios thus become, as far as an explanation of this condition goes, merely of importance insofar as it concerns the speed of the process. Thus if there is a genetic state that produces large numbers of blind fish per generation it would move relatively fast, whereas if the blind individuals are genetically fairly rare it would move more slowly to reach the present state of affairs as found in La Cueva Chica.

The continual locomotor activity and lack of any social grouping on the part of these blind fish would suggest that this feature of behavior has been obliterated, for insofar as experimental evidence goes, schooling is dependent on vision alone in other more or less similarly constituted fishes. Breder & Nigrelli (1935 and 1938) discuss the role of vision in schooling and give full bibliographies.

Since thus far no substitute sensory mechanism has been demonstrated in this form and since nothing in their behavior indicates the presence of such, we infer that these fish are managing to survive in their specialized environment while laboring under a simple loss of function. This may mean that in an evolutionary sense this group is of relatively recent establishment as compared with other blind cave fish that possess elaborate non-visual sensory organs.

Park, Roberts & Harris (1941) have shown that the crayfish of Mammoth Cave, *Cambarus pellucidus* Tellkamp, is light negative in a manner that seems to be fairly comparable to the present findings insofar as it is possible to

compare a sedentary invertebrate to an active fish.

A study of the exact means whereby these fish are enabled to react to the presence of light or on associated phenomena is now being undertaken.

SUMMARY.

1. Fully blind characins from La Cueva Chica are slightly negative to light or some associated effect, as are their fifth generation descendants reared in light.
2. There is a sharp break in the locomotive behavior pattern between those which can form a retinal image and those which cannot.
3. Individuals which form a retinal image have a strong schooling instinct and those which do not, evidence none.
4. Individuals with vision tend to rest quietly in a compact school while the blind continually wander about in an apparently aimless manner.
5. In light, attempts by seeing individuals to school with the blind often terminate by the former killing the latter.
6. Blind individuals in a newly rearranged aquarium will swim into obstacles but will avoid them after a time, apparently by some process of learning.
7. Apparently no substitute mechanism has been developed to function as a substitute for the lost vision.
8. The differential behavior existing between the blind and the seeing is apparently an additional positive factor in the establishment of this cave form.

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EXPLANATION OF THE PLATES.

All fish on the same scale. All eye sections on the same scale.

Photographs of fish by S. C. Dunton. Photomicrographs by M. Sparago.

PLATE I.

- Fig. 1. Unpigmented specimen from La Cueva Chica, Pool No. 2. 53 mm. standard length.
Fig. 2. Section of eye of the above specimen, showing sunken and overgrown eye with cystic development. (Group 1.)

PLATE II.

- Fig. 3. Scarcely pigmented specimen from La Cueva Chica, Pool No. 3. 47 mm. standard length.
Fig. 4. Section of eye of the above specimen, showing no exterior chamber, lens or pupillary area development and retina partially developed. (Group 2a.)

PLATE III.

- Fig. 5. Partially pigmented specimen from La Cueva Chica, Pool No. 3. 58 mm. standard length.
Fig. 6. Section of eye of the above specimen, showing uncovered sunken eye with well developed form, iris poorly developed, retina, choroidal gland and optic nerve undeveloped. (Group 2b.)

PLATE IV.

- Fig. 7. Normal river fish from the Rio Tampaon. 67 mm. standard length.
Fig. 8. Section of eye of the above specimen, showing the normal optic constitution of these fishes. (Indistinguishable from Group 3.)